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ORIGINAL ARTICLE

Assessment of pesticide residues in blood samples of agricultural workers in Egypt

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Abstract

The aim of this study was to monitor pesticide residues in the blood of agricultural workers (farmers, pesticide dealers, and spraying workers) living in the Dakahlia Governorate, Egypt. Residue analysis revealed that 48, 76, and 84% of the farmers, pesticide dealers, and spraying workers had pesticide residues in their bloods, respectively. Eleven compounds were detected in the blood of examined individuals. According to the World Health Organization (WHO) classification, most of these pesticides (nine pesticides) were in moderately hazardous compounds. Carbofuran, a highlyhazardous compound was the most toxic. The compound with the lowest toxicity was hexytiazox, which is unlikely to pose an acute hazard in normal use. Chlorpyrifos was found in the blood of 38.3% of the study subjects, followed by acetamiprid (11.7%) and profenofos (10.7%), while fenvalerate was the lowest occurring compound (1.3%). Of the collected samples 41.3% was free of pesticide residues, while 58.7% of the samples was contaminated. Furthermore, the amounts of all detected pesticides were below the no observable adverse effect levels (NOAEL). Also, 38.7% of the samples had only one pesticide, while 8% of them contained residues of two pesticides, and 5.3% contained more than two compounds. The worker's age did not affect the accumulations of pesticide residues in their bodies. However, there was a strong correlation between pesticide residues accumulation and an individual's exposure time. Therefore, from these results it can be seen that encouraging greater awareness among pesticide users of the need to improve safe usage and handling of pesticides by education, advice, and warning them of the risks involved in the misuse of these poisonous materials is highly recommended.

Keywords: agricultural workers, blood, pesticide exposure, residues

Introduction

Pesticides play a pivotal role in the control of vector-borne diseases and producing food, cotton-fiber, and tobacco required by the escalating population. Most applied pesticides disperse in the environment and affects the health of unprotected industrial and agricultural workers (Imran and Dilshad 2011; Abdel-Daim *et al*. 2014). These chemicals have adverse health effects on agricultural workers based on their structural likeness to toxicants. The excessive and unregulated use of pesticides has become a major bottleneck in the fight against insect pests (Shalaby *et al*. 2012). The low education level of agricultural workers, lack of information and training about pesticide safety, poor spraying equipment, and insufficient personal protection during pesticide use play a major role in pesticide toxicity (Hurtig *et al*. 2003; Atreya 2008). Generally, knowledge of the main determinants of pesticide exposure in developing countries is often poor and exposure situations may differ between countries. Farmers and pesticide applicators are directly exposed to pesticides while mixing and handling the pesticides on crops. Exposure also comes through contaminated soil, air, drinking water, food consumption and smoking at workplaces.

Ultimately these are absorbed by inhalation, ingestion, and dermal contact. In developing countries, agricultural workers are exposed to pesticide contamination due to incorrect application, poor or inappropriate equipment, inadequate storage practices, lack of personal protective equipment and reuse of old pesticide packaging for food or water storage (Ecobichon 2001; Damalas and Eleftherohorinos 2011; Nassar *et al.* 2016). The residue concentrations of these compounds in the exposed spray-workers can lead to a variety of metabolic and systemic dysfunctions and in some cases outright disease (Soomro *et al.* 2008). In 2018, approximately 11,000 t (active ingredients – a.i.) of pesticides (4,510; 4,290 and 2,200 t of herbicides, insecticides, and fungicides, respectively) were used in Egypt according to the Agricultural Pesticides Committee (APC), Egypt (2019). Pesticides misuse together with weak legislation concerning their use, are some of the major reasons for most occurrences of pesticide toxicity in developing countries (Konradsen *et al.* 2003). Hence, the objective of this study was to monitor pesticide residues in blood samples collected from exposed agricultural workers living in the Dakahlia Governorate, Egypt during 2017. The effect of workers' age, and exposure time on pesticide residues was also investigated.

Materials and Methods

This study was carried out on agricultural workers living in the Dakahlia Governorate, Egypt, from February to June 2017. One hundred and fifty healthy male subjects, 30–55-years-old were selected for the present study (100 farmers, 25 spraying workers, and 25 pesticide dealers). These workers had been exposed to different classes of pesticides for at least 5 to 15 years.

Study area

Dakahlia Governorate is located east of the Delta region in northern Egypt (30.45–31.60 N; 31.15–32.00 E), and has a population of approximately 7 million people. Agriculture is the main profession of the area. It is famous for the dense traditional cultivation of a large number of crops (rice, wheat, onions, sugar beets, potatoes, tomatoes, cabbage, alfalfa, corn, etc.). A large number of pesticides are used annually to control different pests which attack these economic crops. The objectives of the experiment were described carefully to volunteers and they signed an informed consent before the collection of blood samples. The study protocol was approved by the human and animal ethics committee of the National Research Centre, Egypt.

Chemicals used

Magnesium sulfate, primary secondary amine (PSA), and sodium acetate were purchased from Agilent Technologies, Cairo, Egypt, Acetonitrile HPLC grade solvent.

Blood sample collection

Blood samples were collected with technical assistance from the Department of Molecular Genetics Enzymology, National Research Centre, Egypt. The skin of each subject was properly cleaned with a swab of methylated spirit to minimize sample contamination from possible insecticide residue adsorbed to the skin. Thereafter, using butterfly needles, blood samples were drawn from the veins in the inner forearms of each subject. Five milliliters of blood was collected and dispensed into decontaminated tubes; all samples were transported in a cold chain to the laboratory. The serum was separated by cooling centrifuge (at –20°C) in decontaminated tubes (Sosan *et al.* 2008).

Pesticide residue analysis

Modified QuECHERS extraction method followed by Gas Chromatography-Mass Spectrometry (GC–MS) was used for the determination of pesticide residues according to Usui *et al.* (2012) and Nassar *et al.* (2016). Supernatants were collected and filtered through 0.22 μm polytetrafluoroethylene (PTFE) filters (Millipore, USA) into 2 ml clear vials. About 1 μl of each sample was injected directly into the GC–MS/MS system.

GC-MS/MS analysis

Determination of pesticide residues was performed at the Central Agricultural Pesticide Laboratory, Egypt. Pesticide standards were obtained from Sigma Chemical Company, USA. Analysis of prepared samples was carried out on the Agilent 6890N GC system coupled with an Agilent 5973 mass – selective detector. Samples were separated on a DB-17 ms capillary column of 0.25 mm i.d., 30 m length, and 0.25 μm film thickness. The chromatographic conditions were those described by (Nassar *et al.* 2016). Analyte identification was performed by comparing both the retention time and the MS spectrum of the sample peaks with those of standard solutions.

Quality control

For the estimation of the accuracy of the analysis method, five replicates of blood samples were spiked with 0.1 mg · l^{-1} of the analytes and processed through the whole analytical procedure. The accuracy of the analytical method was calculated from the areas obtained from the analysis of spiked samples as a percentage of those obtained from the analysis of the standard solution with an equivalent concentration. Limit of detection (LOD) was defined as the concentration of a compound giving a signal-to-noise ratio (S/N) of 3, while the limit of quantification (LOQ) was calculated from S/N ratio of 1 : 10, which were obtained from the measurement of the samples with the lowest concentration level where peaks of determined pesticides were found. Recovery study was undertaken by analysis of spiked samples as described by Lehotay *et al.* (2005) with known concentrations. The mean recovery values were ranged from 93 to 105%, and the data corrected by the rates of the recovery percentages.

Statistical analysis

Data were analyzed by the analysis of variance (one-way classification ANOVA) followed by the least significant difference, LSD at 5% (Costat Statistical Software 1990).

Results and Discussion

Human beings are exposed to pesticides either by occupational (manufacturing/formulation of pesticides and during application in the agricultural fields) or non-occupational routes (pollution of the ecosystem through the food chain). One hundred and fifty blood samples were collected from pesticide occupational workers (100 farmers, 25 pesticide dealers, and 25 sprayers), 30 to 55 years old, working in these fields for at least 5 years and in a good health. From the results in Tables (1–4) and Figures (1–3) it can be seen that 48, 76 and 84% of farmers, pesticide dealers, and sprayers, respectively, had pesticide residues (Fig. 1). Also, 11 pesticides (eight of them in the blood of farmers, nine in market workers, and eleven sprayers) were found and belonged to different chemical groups (organophosphorus, carbamate, pyrethroid, neonicotinoid) (Tables 1 and 3). The results also show that organophosphate insecticide chlorpyrifos was found in 38.3% of the study population, followed by acetamiprid (11.7%) and profenofos (10.7%), while the lowest was fenvalerate (1.3%) (Table 3). The results show that 41.3% of the samples were free of pesticide residues, while, 58.7% of the samples were contaminated with pesticide residues. It was found that 38.7% of the samples contained only one pesticide, while 8% of them contained residues of two pesticides and 5.3% contained more than two pesticides (Table 2). Chlorpyrifos may be the most widely used for control of pests in study region (38.9%). Subjects free from pesticide residues may have used protective equipment before pesticide applications or the detoxification process was caused by e.g. excretion (Usui *et al.* 2012). These results are in accordance with those obtained by Bedi *et al.* (2015), who reported that the presence of endosulfan residues in the blood of individuals with non-occupational and occupational exposure to pesticide spraying was a matter of public health concern. They also reported that an older age, pesticide spraying activities, and non-vegetarian dietary habits were associated with higher levels of pesticide residues. As the health consequences of pesticides, residue levels in human blood were uncertain the residue amounts comparison with no observable adverse effect level (NOAEL) for pesticides according to Anonymous (2004), it was shown

Table 1. The percent of agricultural workers having pesticide residues and the average of residues [mg · kg^{-1} BW] in their blood

Pesticides	ADI [mg · kg^{-1}]	NOAEL [mg · kg^{-1} BW]	Farmers			Pesticide dealers			Pesticide spray workers		
			% [n = 100]	range [mg · kg^{-1}]	average [mg · kg^{-1}]	% [n = 25]	range [mg · kg^{-1}]	average [mg · kg^{-1}]	% [n = 25]	range [mg · kg^{-1}]	average [mg · kg^{-1}]
Chlorpyrifos	0.01	1.0	27	0.01–0.34	0.03	48	0.01–0.18	0.13	40	0031–0.76	0.15
Acetamiprid	0.07	7.1	7	0.01–0.26	0.016	16	0.01–0.78	0.27	12	0.01–0.08	0.04
Profenofos	0.03	0.3	4	0.03–0.18	0.08	12	0.02–0.17	0.07	16	0.26–2.67	0.1
Pirimicarb	0.02	75.0	4	0.01–0.09	0.04	8	0.01–0.02	0.015	8	0.04–0.08	0.06
Carbofuran	0.001	20.0	3	0.01–0.02	0.02	8	0.01	0.01	4	0.02	0.02
Lambda-cyhalothrin	0.02	0.3	6	0.05–0.17	0.06	4	0.07	0.07	4	0.13	0.13
Fenpyroximate	0.01	1.1	0.0	ND	ND	4	0.01	0.01	8	0.01	0.01
Dinotefuran	0.02	NA	4	0.01–0.07	0.02	0.0	ND	ND	4	0.05	0.05
Cyfluthrin	0.04	50.0	0.0	ND	ND	4	0.03	0.03	4	0.01	0.01
Fenvalerate	0.02	250.0	0.0	ND	ND	0.0	ND	ND	4	0.01	0.01
Hexythiazox	0.03	23.1	4	0.01–0.02	0.015	8	0.02	0.02	8	0.01	0.01

ADI – acceptable daily intake; NOAEL – no observable adverse effect level [according to Anonymous (2004)]; ND – non-detected; NA – not available; BW – body weight; n – number of workers

Table 2. The number of analyzed blood samples, free, contaminated, above or below ADI, with one, two and with more two pesticides

Workers	No. of samples	Free samples	Contaminated samples	No. of samples >ADI	No. of samples <ADI	No. of samples with one pesticide	No. of samples with two pesticides	No. of samples with more than two pesticides
Farmworkers	100	52	48	44	15	40	5	3
Pesticide dealers	25	6	19	21	7	12	4	3
Spray workers	25	4	21	19	9	16	3	2
Total	150	62	88	84	31	58	12	8

ADI – acceptable daily intake [according to Anonymous (2018)]

Table 3. Pesticides detected in subjects' blood, WHO classification and percent of each detected in all samples

Common name	Chemical group	WHO category*	Type of use	% detected in samples**
Chlorpyrifos	organophosphorus	II	insecticide	38.3
Acetamiprid	neonicotinoid	II	insecticide	11.7
Profenofos	organophosphorus	II	insecticide/acaricide	10.7
Pirimicarb	carbamate	II	insecticide	6.7
Carbofuran	carbamate	IB	nematicide	5.0
Lambda-cyhalothrin	pyrethroid	II	insecticide	4.7
Fenpyroximate	METI	II	acaricide	4.0
Dinotefuran	neonicotinoid	II	insecticide	2.7
Cyfluthrin	pyrethroid	II	insecticide	2.7
Fenvalerate	pyrethroid	II	insecticide/acaricide	1.3
Hexythiazox	mite growth inhibitor	U	acaricide	6.7

*WHO (2004) classification: IB – highly hazardous, II – moderately hazardous, U – unlikely to pose an acute hazard in normal use
**percent of samples containing pesticide residues

Table 4. The total amounts of pesticide residues detected in study subjects' blood

Pesticides	LOQ [ppm]	Farmers	Pesticide dealers	Pesticides spray workers
Chlorpyrifos	10	0.81	1.56	1.50
Acetamiprid	30	0.112	1.08	0.12
Profenofos	10	0.32	0.21	0.40
Pirimicarb	20	0.16	0.03	0.12
Carbofuran	20	0.06	0.02	0.02
Lambda-cyhalothrin	10	0.36	0.07	0.13
Fenpyroximate	15	0.0	0.01	0.02
Dinotefuran	25	0.08	0.0	0.05
Cyfluthrin	15	0.0	0.03	0.01
Fenvalerate	10	0.0	0.0	0.01
Hexythiazox	30	0.06	0.04	0.02
Total [mg]		1.962	3.05	2.42

LOQ – limit of quantification

that residues of all detected insecticides in all individuals were below the NOAEL. These results agree with those obtained by Sosan *et al*. (2008), who found that residues of lindane, endosulfan, and propoxur were below the NOAEL in the blood of cacao farmers, however, the residue of diazinon exceeded the NOAEL (0.02 mg · kg^{-1}).

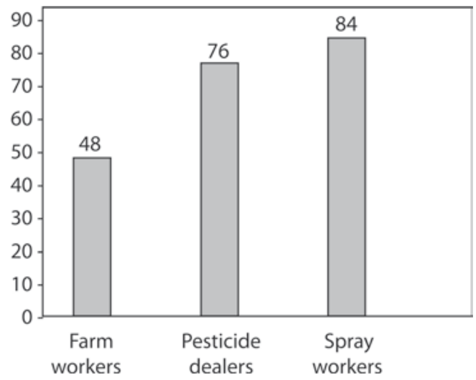

Fig. 1. Percent of agricultural workers having pesticide residues in their blood

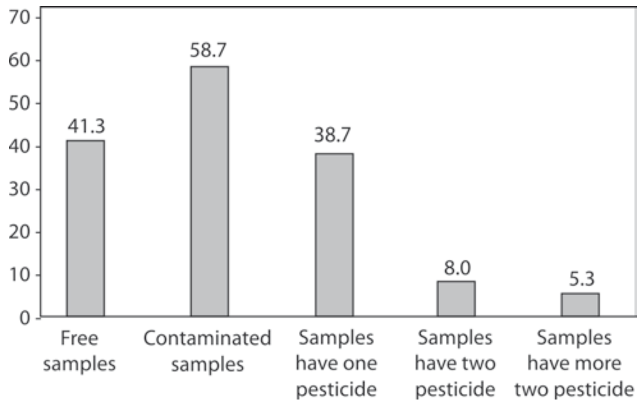

Fig. 2. The percent of blood samples contaminated by pesticide residues

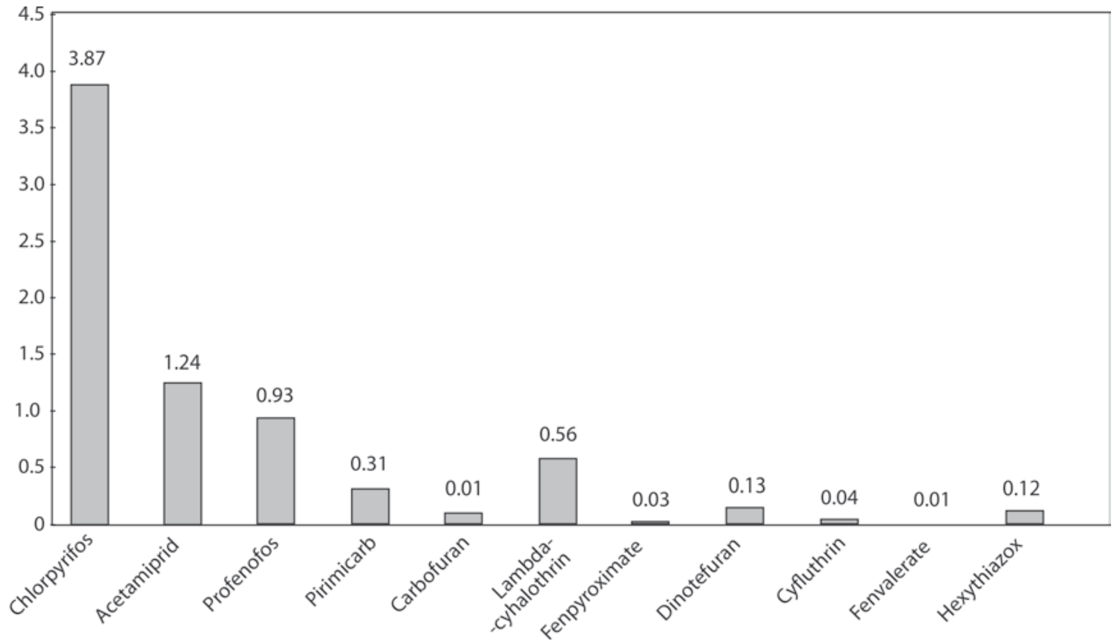

Fig. 3. Total amount of each pesticide residues [mg] in all blood samples

The results can be summarized in the following points:

1. **Farmers**: pesticide residues were estimated in 100 samples; 48 samples contained eight pesticide residues and 52 pesticide-free samples were also found, while 15 pesticides were less than the Acceptable Daily Intake (ADI) (Anonymous 2018). Also, analysis revealed the presence of residues of eight pesticides: chlorpyrifos, acetamiprid, profenofos, pirimicarb, carbofuran, lambda-cyhalothrin, dinotefuran and hexythiazox at an average of 0.03, 0.016, 0.08, 0.04, 0.02, 0.06, 0.02 and 0.015 mg · kg^{-1}, respectively. Profenofos had the highest average, its amounts ranged from 0.03 to 0.18 mg · kg^{-1}. It was detected in four samples of farmer blood. Data show that 40 samples contained residues of one pesticide, five samples contained residues of two pesticides and three samples contained residues of more than two pesticides (Table 2). These results agree with those obtained by Sosan *et al.* (2008), who found that 42 of 76 farmers had residues of lindanein, endosulfan, diazinon, and propoxur in their blood.
2. **Pesticide dealers**: obtained data revealed that nine pesticides residues were detected in 19 samples (76%): chlorpyrifos, acetamiprid, profenofos, pirimicarb, carbofuran, lambda-cyhalothrin, fenpyroximate, cyfluthrin and hexythiazos, averaging 0.13, 0.27, 0.07, 0.015, 0.01, 0.07, 0.01, 0.03 and 0.02 mg · kg^{-1}, respectively. Acetamiprid had the highest average, ranging from 0.01 to 0.78 mg · kg^{-1}, while carbofuran and fenpyroximate had the lowest amounts (0.01 mg · kg^{-1}) and both compounds were detected in one sample. Results also showed

21 pesticides higher than the ADI, while 7 pesticides were less. Twelve samples contained residues of one pesticide, four samples contained two pesticides, and three samples contained more than two pesticides.

3. **Spraying workers**: pesticide residues were found in 25 samples; 11 pesticide residues were detected in 21 samples (84%), of which 19 residues were higher than ADI while nine were less. There were 16 samples containing one pesticide, three samples containing pesticide residues and two samples containing more than two pesticides. Data revealed that profenofos was the highest detected amount (Table 1) in spray workers (2.67 mg · kg^{-1} BW). These results agree with those obtained by Arshad *et al.* (2016) who reported that 72% of all blood samples of workers in pesticide manufacturing industries had malathion residues with a mean value of 0.14 mg · l^{-1} (ranging from 0.01 to 0.31 mg · l^{-1}). Also, Soomro *et al.* (2008) found residues of endosulfan, monocrotophos, carbary, and cypermethrin in blood samples collected from spray – workers living in 14 districts of Sindh province, Pakistan during 2005. Their amounts ranged from 0.009 to 0.08 mg · kg^{-1} BW. Our results agree with those obtained by Tambe *et al.* (2019), who reported that the occupational health safety conditions on tomato farms in Cameroon were mostly poor, thus predisposing farmers to the risk of work-related health problems. Exposure to occupational hazards can be significantly reduced if the required personal protective equipment were available and efficiently used. On the contrary, data obtained by Nassar *et al.* (2016) revealed that no pesticide residues were detected in spray-farmers' blood in the Albeheira Governorate, Egypt during September 2013, except for the herbicide ethofumesate.

   Data in Table 3 revealed that nine of the detected insecticides (chlorpyrifos, acetamiprid, profenofos, pirimicarb, lambda-cyhalothrin, fenpyroximate, cyfluthrin, dinotefuran, and fenvalerate) are WHO (World Health Organization) category II insecticides (moderately hazardous with a median lethal dose (LD_{50}) 50–500 mg · kg^{-1}. They are capable of causing acute toxicity as well as chronic intoxication (WHO 2004). Carbofuran is WHO category IB (highly hazardous) with LD_{50} = 5–50 mg · kg^{-1}. Hexythiazox which is unlikely to pose an acute hazard with normal use (WHO category U) with LD_{50} > 5,000 mg · kg^{-1}. Data in Table 4 revealed that the amount of chlorpyrifos residue was higher in all detected samples (3.78 mg), followed by acetamiprid (1.24 mg) and the lowest was fanvalrate (0.01 mg). The results also showed that the amount of pesticide residue detected was higher in pesticide dealers (3.05 mg), than in spray workers (2.42 mg) and less in farmer (1.962 mg). In the same respect, Banday *et al.* (2012) collected randomly 600 blood samples from inhabitants of Dal Lake hamlets in J & K, India (2008–2010). Chlorpyrifos was detected in 82.2% of blood samples (197 males and 214 females) with a mean concentration of 0.5194 ± 0.6456 ng · μl^{-1}. It was detected in 49 (49%) of the control group with 26 males (53.1%) and 23 females (46.9%) with a mean concentration of 0.0008 ± 0.0009 ng · μl^{-1}. Huen *et al.* (2012) reported that levels of diazinon and chlorpyrifos pesticides ranged from 0.0–0.5 ng · μl^{-1} and from 0.0–1.726 ng · μl^{-1}, respectively, in the umbilical cord plasma of women living in an agricultural region.

4. **Effect of workers' age on pesticide residues:** study subjects were classified into three groups according to their age: 1) 30–40-years-old (63 individuals); 2) >40–50-years-old (50 individuals); 3) >50–55-years-old (37 individuals). Data in Table 5 show that the older individuals (>50–55-years-old) had greater pesticide residues amounts than the younger individuals (the averages were 0.135 and 0.162 mg · kg^{-1}, respectively). The differences between the detected amounts in all groups were negligible. This means that the workers' age did not affect the accumulations of pesticide residues in their bodies. Abu Mourad (2005) reported that younger Palestinian farm workers were more affected by insecticides.

5. **Effect of exposure time on pesticide residue:** workers were classified into three groups according

Table 5. Effect of workers' age on pesticide residues

Age	n	Range [mg · kg^{-1} BW]	Average [mg · kg^{-1} BW]
30–40 y	63	ND–0.78	0.136 a
>40–50 y	50	ND–2.67	0.144 a
>50–55 y	37	0.01–0.76	0.166 a
$LSD_{0.05}$		0.068	

n – number of individuals; BW – body weight; ND – non-detected; y – years

Table 6. Effect of exposure time on pesticide residues

Groups	n	Range [$mg \cdot kg^{-1}$ BW]	Average [$mg \cdot kg^{-1}$ BW]
5–10 y	75	ND–0.68	0.244 c
>10–15 y	55	0.01–0.73	0.386 b
>15 y	20	0.01–2.67	0.726 a
$LSD_{0.5}$		0.137	

n – number of individuals; BW – body weight; ND – non-detected; y – years

to time exposed to pesticide residues: 1) 5–10 years (75 individuals); 2) >10–15 years (55 individuals); 3) >15 years (20 individuals). Data in Table 6 show that there was a strong correlation between pesticide residues accumulation and an individual's exposure time. Results showed significant differences in detected residue amounts between the different groups. The average of detected residues in individuals exposed to pesticides for 5–10 years was 0.244 $mg \cdot kg^{-1}$. This amount increased to 0.386 $mg \cdot kg^{-1}$ for individuals exposed >10–15 years and reached 0.726 $mg \cdot kg^{-1}$ for individuals working more than fifteen years in agricultural activities. Obtained results indicated that most of the detected pesticides are used on a large scale to control pests, while previous studies revealed that these had a less persistence under different conditions, which can be seen in the low accumulation in the blood of agricultural workers by increasing the exposure period as mentioned by Jaga and Dharmansi (2003) and Shalaby *et al*. (2018). In the same respect, Khalaf-Allah (1999) reported that the exposure period and an older age of workers caused an increase in white blood cells (WBCs) count, which is a clear indicator of damage to DNA and blood cells. Also, Sosan *et al*. (2008) observed that 47.6% of cacao farmers in southwestern Nigeria with pesticide residues in their blood had been involved in insecticide application for over 20 years while 23.8% and 21.4% had been involved in insecticide application for 10–14 years and 5–9 years, respectively. Therefore, it can be seen that there is a trend in residues accumulation in the blood of the workers that seems dependent on the exposure time.

The hazard of pesticide exposure can be acute or chronic. Their effects vary depending on the toxicity of concentration, active ingredients, exposure time and an individual's health status (Bonmatin *et al*. 2015; Zikakuba *et al*. 2019).

Conclusions

It can be concluded that pesticide workers may be occupationally exposed to pesticides during pesticide application for pest control on their farms. The exposure at times can be of such magnitude as to be risky to them and their respective communities. Intensive training and efforts are necessary to build awareness of essential safety practices and to change workers' behavior that possibly may be harmful to the environment. Also, the role of government regulations and agricultural research services with the collaboration of pesticide manufacturers is very important for reducing pesticide hazards and risks.

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